REMARKS

Claim Amendments

Upon entry of this amendment, claims 401, 411, 414, 416, 419, 422-424, 464-465, 469-471 and 478-480 will be pending in the application.

Independent Claims 401 and 411 have been amended to recite that the culture medium includes each of stem cell factor, thrombopoietin, FLt3 ligand, and IL-6 and optionally IL-3. Support for this amendment is found throughout the specification (*See*, *e.g.* page 25, lines 24-28). Claims 401 and 411 have also been amended to recite that the observed greater percentage of $CD34^+/CD38^-$ and $CD34^+/Lin^-$ cells can be observed after a three week culture period. Support for this amendment can be found in Example 5 of the specification as filed.

No new matter is added. The amendments to the claims are solely for the purpose of clarity and do not require the Examiner to conduct a new search – thus, applicant requests that these claim amendments be entered.

The Claims Are Not Obvious over Brown in View of Block

There is a single outstanding obviousness rejection in this case – the Examiner has rejected the claims over a combination of Brown (US Publication 2002/0159984) in view of Block (US 6,413,772). Applicants traverse.

On page 3 of the Office Action, the Examiner contends that the phrase "a combination of cytokines including stem cell factor, thrombopoietin, FLt3 ligand, IL-6 and optionally IL-3" can be construed to permit any combination of the recited cytokines. Applicants disagree. However, so there can be no doubt, applicants have amended the claims to make crystal clear that the growth conditions require each of stem cell factor, thrombopoietin, FLt3 ligand, and IL-6 (and optionally IL-3). Brown simply does not teach that the use of these four required cytokines together in the presence of nicotinamide within the claimed range will produce hematopoietic cell populations with a greater percentage of $CD34^+/CD38^-$ and $CD34^+/Lin^-$ cells after a three week culture period. In fact, Brown is fatally deficient -- Brown shows exactly the opposite result. Figure 3 of Brown clearly demonstrates that after Day 14, the percentage of $CD34^+/CD38^-$ cells declines (whether in the presence or absence of serum). For this reason alone, the obviousness rejection fails.

The Examiner has also dismissed the February 2010 Declaration of Dr. Tony Peled, allegedly on the ground that no statistical analysis was presented. The statements in paragraphs 4 and 5 are evidence of record that cannot be ignored -- Applicants reiterate those conclusions here. Specifically, Dr. Peled's February 2010 Declaration stated that her data:

> "demonstrates that culturing in a growth media including stem cell factor, thrombopoietin, FLt3 ligand and IL-6 in the presence of serum and nicotinamide results in a cell population that is expanded in the population of CD34+ hematopoietic stem cells with an increased proportion of CD34+/Lin- and CD34+/CD38- cells in the expanded culture as compared to CD34+ cells cultured in the presence of cytokines and nutrients without exogenously added nicotinamide." February 2010 Decl. Of Dr. Peled, ¶ 4.

For the avoidance of doubt, Applicants have also submitted a further Declaration of Dr. Tony Peled under 37 CFR §1.132 ("July 2010 Peled Decl.") which sets forth data confirming that the specifically claimed combination of cytokines produces an expanded CD34+ hematopoietic stem cell population with an increased proportion of CD34+/CD38- and CD34+/Lin- cells in the expanded culture as compared to CD34+ cells cultured in the presence of those cytokines and nutrients without exogenously added nicotinamide (as expressly recited in independent claims 401 and 411 – and thus all the claims that depend therefrom). Furthermore, Dr. Peled's July 2010 Declaration makes crystal clear that the results are statistically significant. In addition, the Examiner is directed to Example 5 of the specification as filed which provides further evidence of the unexpected results obtained according to the claimed methods and cell populations obtained thereby.

The combination of Brown and Block teaches away from the claimed invention. The Examiner concedes that Brown is fatally deficient in failing to disclose the claimed range of nicotinamide concentration. Brown does not suggest to the skilled artisan that the claimed nicotinamide concentration range in serum free media can act as an agent that maintains CD34+ hematopoietic cells in an undifferentiated state and enriches for CD34+/CD38- and CD34+/Lin- cells while the cells are expanded in *ex vivo* culture using a serum-containing culture medium – as claimed here.

Block does not cure the deficiencies of Brown – Block teaches away. Block refers to the use of nicotinamide in the culture/expansion of differentiated hepatocytes – a completely different cell population than the claimed CD34+ hematopoietic stem cell population. Further, none of the cytokines recited in instant claims are present in the Block culture medium. And the ordinarily skilled artisan would not select (out of the many "ingredients" in Block's culture

medium) nicotinamide for use as claimed here. To the contrary, Block teaches the exact opposite – in Block, nicotinamide was used to maintain the differentiated state of the hepatocytes (exactly opposite to the use in the currently claimed invention). *See*, Block, col. 8, lines 26-28. Block also directly teaches away from the use of serum in the culture – as expressly recited in the claims here. The entire focus of Block is to provide a chemically defined culture medium that is serum free. *See*, *e.g.*, col. 1, lines 43-50 and col. 4, lines 8-10.

Applicants reiterate the statement in their February 22, 2010 Response, that hepatocytes are a completely different cell population from undifferentiated CD34+ hematopoietic stem cells. Block discloses the use of nicotinamide for maintaining differentiated hepatocytes in culture. Here the invention recites methods and compositions of cell that are enriched for undifferentiated cells (as evidenced by the increased proportion of CD34+/Lin- cells and the increased proportion of CD34+/CD38- cells in the culture) after 3 weeks in the presence of the recite dcytokines and nictinamide. That is the opposite to the teachings of Block.

One of ordinary skill in the art combining the teachings of Brown and Block would not, and could not, reach the present invention with predictable results.

The data presented herewith in the July 2010 Peled Decl. and in both previously submitted February 2010 Peled Decl. (see specifically ¶¶ 4-6, Figures 1 and 2) and 2008 Peled Declaration (pages 2-4 and Figure 1) shows that using nicotinamide in the range of 1.0 mM to 10 mM, as required by the instant claims inhibits differentiation of $CD34^{+}$stem cells (as evidenced by the unexpectedly and substantially increased cell density of undifferentiated CD34+/CD38- and CD34+/Lin- cells), while permitting expansion, *ex vivo*. These unexpected and superior properties are not taught or suggested by the prior art.

CONCLUSION

Applicants submit that the application is in condition for allowance and such action is respectfully requested. However, if upon receipt and review of this amendment, the Examiner believes that the present application is not in condition for allowance and that changes can be suggested which would place the claims in allowable form, the Examiner is respectfully requested to call Applicant's undersigned counsel at the number provided below.

Respectfully submitted,

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